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Biotransformation of the Insecticide Lindane by the White rot basidiomycete *Phanerochaete chrysosporium*

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Abstract: Biotransformation of the insecticide lindane by the white rot basidiomycete *Phanerochaete chrysosporium* has been investigated in liquid cultures. Some polar metabolites and carbon dioxide were produced from the pesticide. Among the metabolites identified were tetrachlorocyclohexene, tetrachlorocyclohexene epoxide and tetrachlorocyclohexenol. When used as a substrate, tetrachlorocyclohexene was also converted by the fungus to tetrachlorocyclohexenol, polar metabolites and carbon dioxide. Three incubation conditions leading to low and high peroxidase production were assayed. Data from these experiments, as well as in-vitro incubations with purified enzymes, ruled out any involvement of the peroxidases in lindane biotransformation and mineralization. Moreover, 1-aminobenzotriazole (a P450 inactivator) drastically reduced pesticide metabolism. Conversely, phenobarbital (a P450 inducer) did not significantly increase lindane breakdown.

Key words: basidiomycete, lindane, biotransformation, metabolites, P450 monooxygenase.

1 INTRODUCTION

The insecticide lindane (**1**, Fig. 1; 1α,2α,3β,4α,5α,6β-hexachlorocyclohexane, γ-HCH) has been extensively used worldwide, despite its persistence in the environment, tendency toward bioaccumulation and toxicity to higher animals, as well as possible toxicological and environmental problems of its residues.[1,2] It is still used in France for agricultural and medical purposes, and residual pesticide has been detected in living organisms,[3] soils and surface waters.[4] Thus, the microbial metabolism of lindane remains a current subject of investigation.[5–9]

The use of micro-organisms for bioremediation requires an understanding of all the physiological, microbiological and biochemical aspects involved in pollutant transformation. For many years, the white rot basidiomycete *Phanerochaete chrysosporium* Burdsall has been a useful agent for liquid effluent and soil bioremediation. The extended biodegradative properties of the fungus toward numerous environmentally persistent chemicals[10,11] have been credited to its lignin-degrading system (LDS), which includes mainly extracellular lignin peroxidases (LiPs) and manganese-dependent peroxidases (MnPs).[12] The enzymes are produced under substrate-limiting growth conditions, and they are not induced by pollutants. The extracellular peroxidases can also act on compounds with low water solubility, and/or adsorbed on solid particles. Nevertheless, the mechanisms by which white rot fungi degrade pollutants are known for only a few models.

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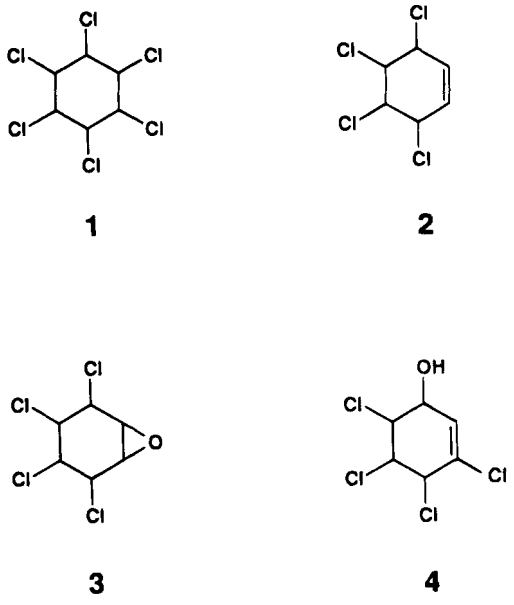

Fig. 1. Chemical structure of the chemicals cited in the text. **1**, lindane (γ-HCH); **2**, tetrachlorocyclohexene (TCCH); **3**, tetrachlorocyclohexene epoxide (TCCE); **4**, tetrachlorocyclohexenol (TCCOL).

P. chrysosporium cultured under ligninolytic conditions has been reported to partially mineralize lindane in liquid cultures,[13,14] and in corncob-amended silt loam soil.[14] Moreover, Shah *et al.*[11] suggested that the LDS could transform the pesticide, without showing any direct proof. Today, little is known about the physiological or biochemical mechanisms occurring in lindane biotransformation by the fungus.

In the present paper, we report the first characterization of the main metabolites formed from lindane by liquid cultures of *P. chrysosporium*. We also attempt to shed some light on the biochemistry of lindane transformation by the fungus. For that purpose, we have assayed lindane transformation by purified peroxidases, run cultures producing low and high ligninolytic activities, and studied changes in insecticide transformation in response to P450 modifiers.

2 EXPERIMENTAL METHODS

2.1 Fungus

Phanerochaete chrysosporium BKM-F-1767 (ATCC 24725) was used. The strain was maintained on malt agar slant cultures at 37°C. Spores were collected by washing slants with sterile distilled water and were stored at 4°C.

2.2 Chemicals

High-purity standards of lindane were obtained from Cluzeau Info Labo (Sainte Foy la Grande, France). [^{14}C]Lindane (1191 MBq mmol^{-1}, radiochemical purity >99·5%) was obtained from Rhône-Poulenc Agrochimie (Lyon, France). [^{14}C]Tetrachlorocyclohexene (1191 MBq mmol^{-1}, radiochemical purity >99·5%) was obtained by [^{14}C]lindane dehalogenation according to a described protocol.[1] It was purified and analyzed by HPLC. Its structure was checked up by mass spectral analysis. The analytical procedures are described in the following sections.

Chemical structures of lindane and its metabolites identified in this study are presented in Fig. 1.

2.3 Incubation conditions for liquid cultures

Spores were inoculated in 150-ml Erlenmeyer flasks containing a previously described growth medium (10 ml) in the presence of 0·06 g litre^{-1} veratryl alcohol.[15,16] The medium was supplemented with labelled lindane or tetrachlorocyclohexene (2·7 nmol ≡ 0·27 μM; 3·3 kBq) dissolved in acetone (10 μl). The cultures were incubated without shaking in total darkness. Three incubation protocols were used. (A) The Erlenmeyer flasks were sealed with cotton stoppers and incubated in one-litre sealed flasks in the presence of vials containing of sodium hydroxide (1 M; 10 ml, in order to trap carbon dioxide) and water (10 ml, to minimize evaporation of the medium). A third vial containing 2-methoxyethanol (10 ml) was added to some flasks to trap organic volatiles. The headspaces of the flasks were flushed with air for 1 min at the beginning of the experiments, and then every four days. The sodium hydroxide solutions were also replaced every four days. Culture temperature was 25°C. This protocol was routinely used in this work. (B) The same experimental procedure was used at 37°C. (C) The Erlenmeyer flasks were tightly sealed with rubber stoppers after they were flushed with pure oxygen for 1 min. The stoppers were connected via manifolds to carbon dioxide traps consisting of Carbomax cocktail (7 ml; Lumac, Landgraaf, The Netherlands). Carbon dioxide was displaced for 10 min from the cultures into the traps by a stream of sterile air at a flow rate of 30 ml min^{-1}. The culture temperature was 37°C. The A,B,C incubation protocols were designated 25°-air, 37°-air and 37°-oxy, respectively. Protocol C was typically used to produce high levels of LiPs and MnPs,[15,17,18] whereas the two former were intended to reduce peroxidase production.

Culture media and mycelia were separated every two days by filtration on Whatman GF/A glass-fibre filters (Prolabo, Paris, France) layered on a 1·2 μm cellulose membrane (Millipore, St Quentin Yvelines, France). The filtration system was subsequently rinsed by Milli-Q water (10 ml), and the rinsing fraction was pooled with its corresponding culture medium. The radioactivity of the trapped carbon dioxide was measured by liquid scintillation counting.

Studies were also undertaken to determine if P450s (heme-thiolate monooxygenases) are involved in lindane

biotransformation. For that purpose, we assayed the ability of P450 modifiers to alter the pesticide transformation rate in *P. chrysosporium* cultures under 25°-air conditions. The modifiers were: 1-aminobenzotriazole (ABT), a classical mechanism-based inactivator of P450s from mammals[19] and higher plants[20] and phenobarbital (PB) an inducer of P450s in higher plant tissues.[21] The chemicals were added to the cultures in acetone (100 μl) without any effect of the solvent on fungal growth. Controls were supplemented with a same volume of acetone, and all samples were analysed four, eight and 12 days after treatment.

In all experiments, uninoculated sterile media were maintained as controls.

2.4 Enzymatic assays with crude extracellular fluid

LiP activity was determined from the rate of oxidation of veratryl alcohol to veratraldehyde at 30°C as described by Tien and Kirk.[22] Assays were performed with culture fluid (400 μl). MnP activity was also determined spectrophotometrically by the method of Paszczynski *et al.*[23] with vanillylacetone as a substrate. Assays were performed with extracellular fluid (100 or 200 μl).

Enzymatic activities were expressed in nanokatals: 1 nkat ml^{-1} is equivalent to 60 U $litre^{-1}$.

2.5 Analytical procedures for pesticide compounds

Filtered medium fractions (15 ml) were concentrated on a C_{18} guard column MCH-10 (3 cm × 4 mm ID; Varian, Les Ulis, France) at a flow rate of 1 ml min^{-1} with an isocratic pump (Varian 9001). Elution of labelled compounds was then achieved onto the analytical column ODS-80TM (25 cm × 4·6 mm ID; Varian) with a Varian 9010 pump delivering a solvent system composed of acetonitrile and water, each acidified with phosphoric acid (0·5 g $litre^{-1}$). Elution began with 1% acetonitrile for 3 min, followed by a linear increase to 100% acetonitrile over 15 min, and a stationary phase of 10 min. The radioactivity of the column eluate was monitored by an HPLC LB 507 A radioactivity monitor (EG&G, Evry, France). UV absorbance was also monitored with a variable wavelength detector (Varian 9050).

To analyse the radioactivity in the mycelium, filtered fungal pellets were homogenized in acetonitrile + water (7 + 3 by volume, 5 ml) for 1 min. The homogenate was diluted with water (5 ml) and extracted with dichloromethane (3 × 12·5 ml). After concentration of the organic phase and dissolution in methanol (500 μl), aliquots (100 μl) were injected in HPLC through a 7125 Rheodyne valve. The elution of labelled compounds was achieved using the conditions described above.

The total radioactivities in the cell biomass were determined by combustion in a model 307 oxidizer (Packard, Rungis, France).

2.6 Mass spectral analysis

Lindane metabolites were firstly resolved by HPLC analysis of the growth medium and then collected separately. Acetonitrile contained in the eluate was evaporated under a stream of nitrogen, and the remaining acidic aqueous fraction was extracted by diethyl ether. After concentration, Electron-Impact (EI, 70 eV) mass spectral analysis (GC-MS) was performed on a Nermag (Quad Service, Argenteuil, France) quadrupole R 10-10C analyser piloted by a SIDAR acquisition system. Samples (2 μl aliquots) were introduced by GC on a Girdel serie 32 chromatograph. The gas chromatographic conditions were as follows: BPX-5 (SGE, Villeneuve St-Georges, France) capillary column, 25 m × 0·32 mm ID with 0·25 μm film thickness; carrier gas, helium at 69 kPa; temperature programme 100–240°C, 10°C min^{-1}; Ross Injector temperature, 240°C.

2.7 Fungal biomass determination

Growth was measured in terms of the mycelial dry weight. The filtered mycelium was dried for one day at 90°C.

2.8 Experimental error

Each experiment was done in triplicate and repeated twice. Results are expressed as means. The standard deviation was less than 10% of the mean.

3 RESULTS

3.1 Characterization of some lindane metabolites formed by *Phanerochaete chrysosporium*

The amounts of radioactivity present in the culture medium, in the cell biomass and evolved as carbon dioxide were followed in liquid cultures of *P. chrysosporium* maintained under 25°-air conditions, and are shown in Fig. 2A. During the mycelium growing phase (10 days), the radioactivity detected in the mycelium increased linearly with time to reach 49·6% of the initial radioactivity. Consequently, the radioactivity measured in the medium decreased. A slight but significant decrease of the radioactivity was noticed in the mycelium between days 10 and 14. About 3·9% of the initial

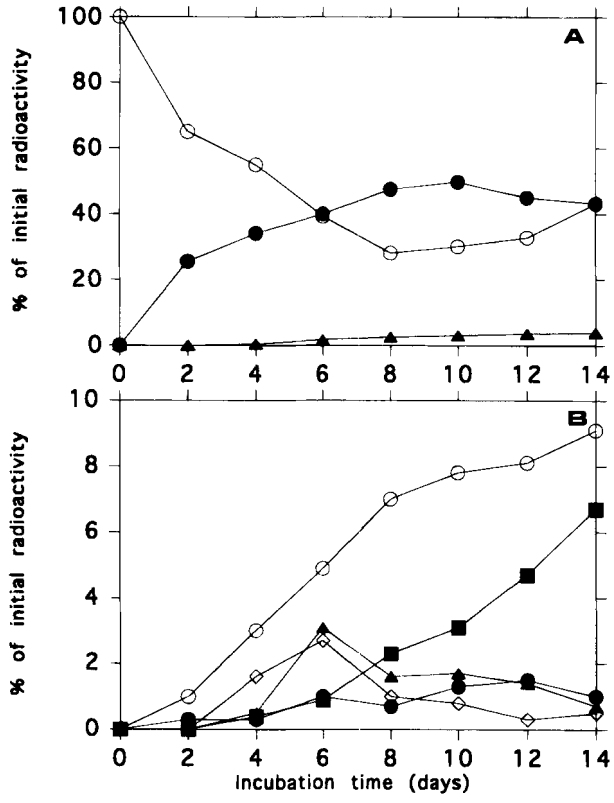

Fig. 2. Profiles of (A) mass balance analysis and (B) metabolite content in liquid cultures of *Phanerochaete chrysosporium* in the presence of [^{14}C]lindane. (A) (○) medium, (●) mycelium (▲) [^{14}C]carbon dioxide; (B) (○) unretained fraction (●) minor metabolites + *b* (■) metabolite *a* (▲) metabolite *c* (◇) metabolite *d*.

radioactivity was trapped as [^{14}C]carbon dioxide after 14 days of culture. At this time, the total radioactivity recovered amounted to 81·0% of the initial radioactivity. This poor recovery is mainly due to lindane volatilization, because radioactivity was found in the traps for organic volatiles in control flasks.

Then, changes in metabolite contents were monitored in cultures of *P. chrysosporium*. Only lindane was extracted from the mycelium. The efficiency of the extraction protocol, evaluated by combustion of other mycelia, was up to 95%. The analysis of the radioactivity in the culture media evidenced a fraction not retained by the guard column. It amounted to 9·1% of the initial radioactivity after 14 days (Fig. 2B). The radioactivity in that aqueous fraction was not extracted by organic solvents, such as chloroform or diethyl ether, suggesting that it included very polar compounds. Attempts to resolve them after concentration of the aqueous phase and injection by the Rheodyne valve were also unsuccessful. By contrast, the radioactivity retained on the guard column was resolved into residual lindane and four main metabolites more polar than the pesticide parent. These metabolites, called *a*, *b*, *c*, *d*, exhibited retention times of 16·5, 17·4, 18·1 and 19·5 min, respectively. The main compounds, *c* and *d*, showed a joint evolution with a maximal amount at day 6, followed by a decrease in the medium (Fig. 2B). Another degradation product, metabolite *a*, represented 6·7% of the initial radioactivity after 14 days, and accumulated during the experiment. A last fraction including minor metabolites and metabolite *b* never exceeded 1·5% of the initial radioactivity. No physicochemical degradation of the pesticide occurred in uninoculated sterile controls.

Higher amounts of metabolites were obtained from *P. chrysosporium* cultures treated with 5·1 μM of lindane. Veratryl alcohol (a component of the growth medium[15,16]) was omitted since this compound is a persistent contaminant during metabolite purification and analysis processes. Lindane transformation was also noticed under that culture condition. The metabolites were collected and analysed separately. The structure of the most polar compound (metabolite *a*) cannot be proved, because of poor GC chromatography behaviour. Attempts to analyse it by direct introduction in the mass spectrometer were also unsuccessful because of the small amount of material. A similar problem occurred for metabolite *b*.

GC-MS mass spectra of the less polar metabolite *d* showed dissociation patterns with fragment ions of *m/z* 183, 147, 122 and 111 (Fig. 3), containing respectively three, two, two and one chlorine atoms. Although molecular ion (*m/z* 218, based on ^{35}Cl) was very low in EI mode, the fragmentation pattern was identical to that of the tetrachlorocyclohexene (TCCH),[1,24] ion at *m/z* 122 corresponding to an anti-Diels-Alder mechanism.

GC-MS analysis of metabolite *c* showed the presence of two different compounds with distinct retention times (c_1: 4·15 and c_2: 4·33 min, respectively) under the peak collected by HPLC. Spectra of compounds c_1 and c_2 (Fig. 3) showed intense cluster ions of *m/z* 199, 163 and 128, corresponding to the step-by-step loss of chlorine atoms from a starting molecule containing four chlorine atoms. The main difference between the two compounds consisted in an intense fragment ion at *m/z* 138 (certainly due to an anti-Diels-Alder reaction) present in the c_2 spectrum and totally non-existent in the c_1 spectrum. Moreover, spectra of compounds c_2 and *d* exhibited a similar pattern, but with a difference of 16 amu due to an oxygen atom for ions at *m/z* 138 (122 + 16), 163 (147 + 16) and 199 (183 + 16). This allowed us to identify this compound (c_2) as an isomer of tetrachlorocyclohexenol (TCCOL) with an allylic hydroxyl group.[6,25] Common features of c_1 and c_2 spectra suggested a tetrachlorocyclohexene epoxide (TCCE) structure for the former (c_1). That hypothesis was in agreement with the chromatographic properties of epoxide and hydroxylated compounds by GC analysis,[25] and with the mechanism of TCCOL formation from TCCH involving attack of oxygen followed by the double bond migration.[8,25]

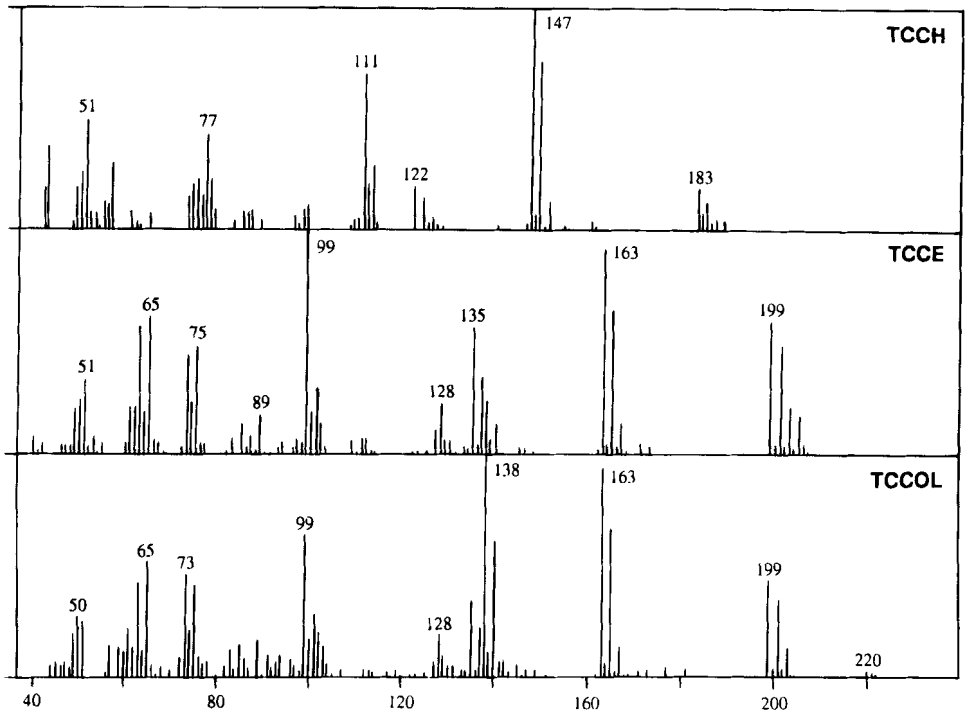

Fig. 3. Mass spectra of compounds identified as TCCH (metabolite *d*), TCCE, (metabolite c_1) and TCCOL (metabolite c_2).

3.2 Tetrachlorocyclohexene transformation by *Phanerochaete chrysosporium*

We studied also the transformation of TCCH by liquid cultures of *P. chrysosporium* to confirm the identity of the main metabolites described. Figure 4A shows that the radioactivity divided between the culture medium and the fungal biomass. It was less abundant in the latter compartment (9·4% of the initial radioactivity after 12 days) by comparison with incubations in presence of lindane (49·6%, Fig. 2A). A lag phase of four days was noticed before the beginning of mineralization, and labelled carbon dioxide represented 7·1% after 12 days of culture. Total radioactivity decreased during the experiment and amounted finally to 62·0% of the initial radioactivity. These results showed an important loss of radioactivity due to volatilization of TCCH and related degradation products.

TCCH content decreased quickly in the culture medium. Conversely, some polar degradation products called α, β and χ were produced. They exhibited retention times of 16·2, 17·2 and 18·1 min, respectively. Their evolution is shown in Fig. 4B. Extracts from incubations with TCCH and lindane have been co-chromatographed. Retention times of the peaks attributed to the most apolar metabolite χ corresponded well to those of the mixture TCCE + TCCOL. Conversely, metabolites α and β did not coelute with metabolites *a* and *b* from lindane incubation extracts. No degradation occurred in uninocolutated sterile controls.

3.3 Effects of incubation conditions on peroxidase production by *Phanerochaete chrysosporium* cultures

The involvement of the LDS in lindane degradation was firstly assayed by using purified LiPs and MnPs[17] exhibiting high levels of veratryl alcohol and vanillyacetone oxidases (83·0 and 195·0 nkat ml^{-1}, respectively). Lindane was not transformed by purified enzymes alone or in mixture under oxidizing conditions (data not shown) nor reducing conditions.[11] In both cases, the addition of culture medium to reaction mixtures, in order to initiate possible co-reactions[12] was also unsuccessful.

LDS production was measured in cultures grown in the presence of lindane, and under the three culture conditions previously defined. Figure 5 shows that the production of LiPs and MnPs is affected by culture conditions. Among the three incubation conditions used, only the 37°-oxy allowed the maximal production of the LDS. In that case, after a one-day lag phase, LiP production increased to its maximal value after four days of culture, and LiP activity reached 16 nkat ml^{-1} (Fig. 5A). Then, it decreased until the end of the experiments. Under the 37°-air condition, the maximal production occurred at day 5, and amounted to 50% of the previous value. The production profile obtained at 25°-air was quite similar to that obtained under 37°-oxy, with a maximum production at day 6.

A similar pattern occurred for MnP production (Fig. 5B). Nevertheless, the lack of pure oxygen was detrimental to the substantial production of enzymes at

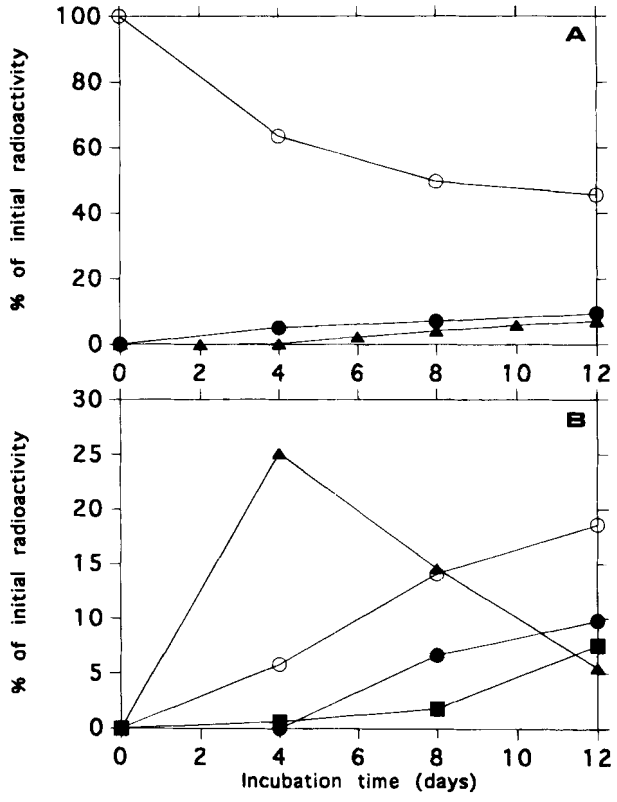

Fig. 4. Profiles of (A) mass balance analysis and (B) metabolite content in liquid cultures of *Phanerochaete chrysosporium* in the presence of [^{14}C]TCCH. (A) (○) medium (●) mycelium (▲) [^{14}C] carbon dioxide; (B) (○) unretained fraction (●) minor metabolites + β (■) metabolite α (▲) metabolite χ.

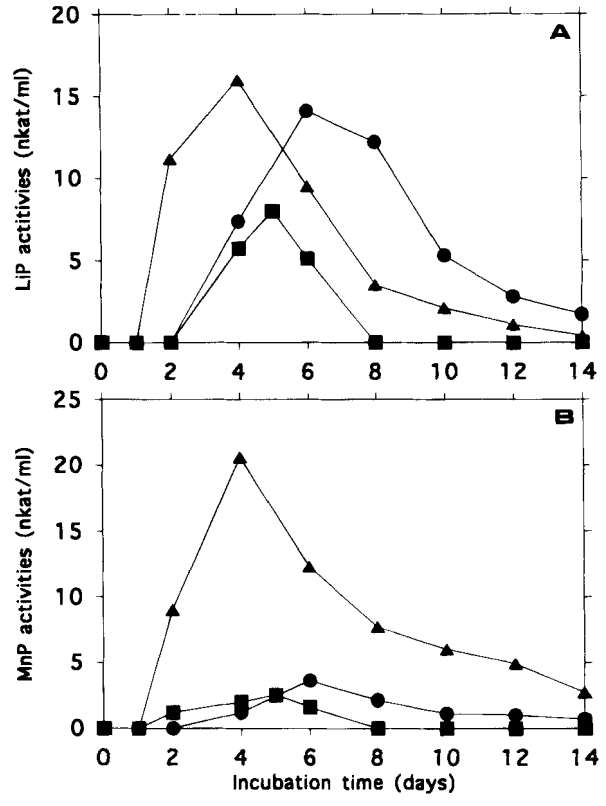

Fig. 5. Profiles of (A) lignin and (B) manganese-dependent peroxidase production by *Phanerochaete chrysosporium*. (●) 25°-air (■) 37°-air (▲) 37°-oxy.

37°C, whilst their production was drastically reduced under 25°-air.

3.4 Effects of incubation conditions on lindane mineralization and degradation by *Phanerochaete chrysosporium*

Lindane mineralization was followed in the same incubations. The results are reported in Fig. 6A. Whatever the incubation conditions, mineralization of lindane occurred, and constituted between 3·0 and 4·5% of the initial radioactivity after 14 days of culture. It began after two days of culture at 37°C, but the lag phase was more pronounced at 25°C. No mineralization was noted in uninoculated sterile controls.

The effects of culture conditions on total metabolite formation were also followed, taking into account both the retained and unretained fractions resulting from HPLC concentration. Total metabolite formation appeared to be quite similar when the fungus was cultured under 37°- and 25°-air conditions (Fig. 6B). The amount of metabolites represented 18·2–18·8% of the initial radioactivity after 14 days of culture, whereas 15·1% were found under 37°-oxy. No degradation was noticed in uninoculated sterile controls. Comparable

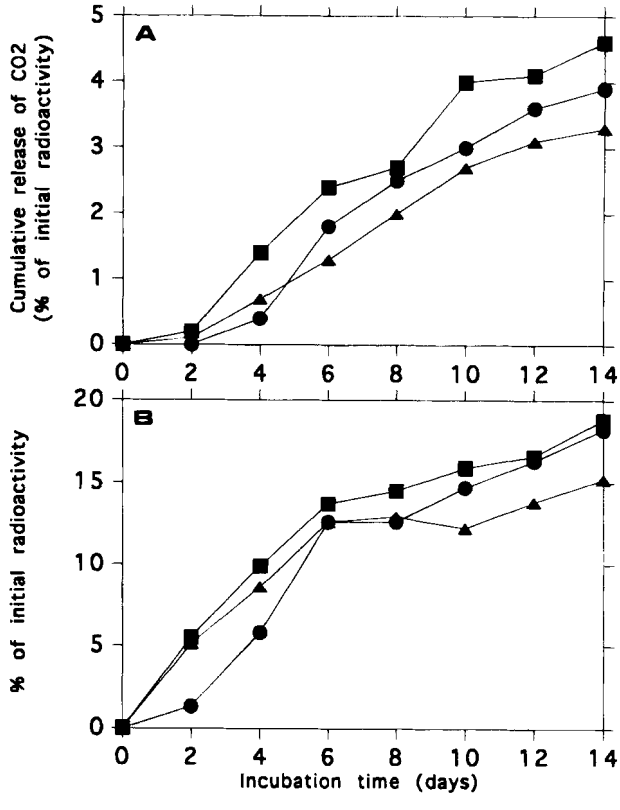

Fig. 6. (A) Mineralization and (B) total metabolite formation from [^{14}C]lindane by *Phanerochaete chrysosporium*. (●) 25°-air (■) 37°-air (▲) 37°-oxy.

changes in separate metabolite amounts occurred under the three culture conditions.

3.5 Effects of P450 modifiers on lindane transformation in cultures of *Phanerochaete chrysosporium*

When applied to fungal cultures at 10^{-6} to 10^{-3} M, ABT inhibited lindane mineralization (Fig. 7A) after four, eight and 12 days of incubation. The inhibition increased with ABT concentration. In a similar way, residual lindane amounts in the medium increased in response to ABT treatment to reach 178·4% of the untreated controls (Fig. 7B). ABT 10^{-4} M drastically reduced the formation of all metabolites in the retained and unretained fractions resulting from medium concentration, which was totally inhibited at the highest modifier concentration (Table 1).

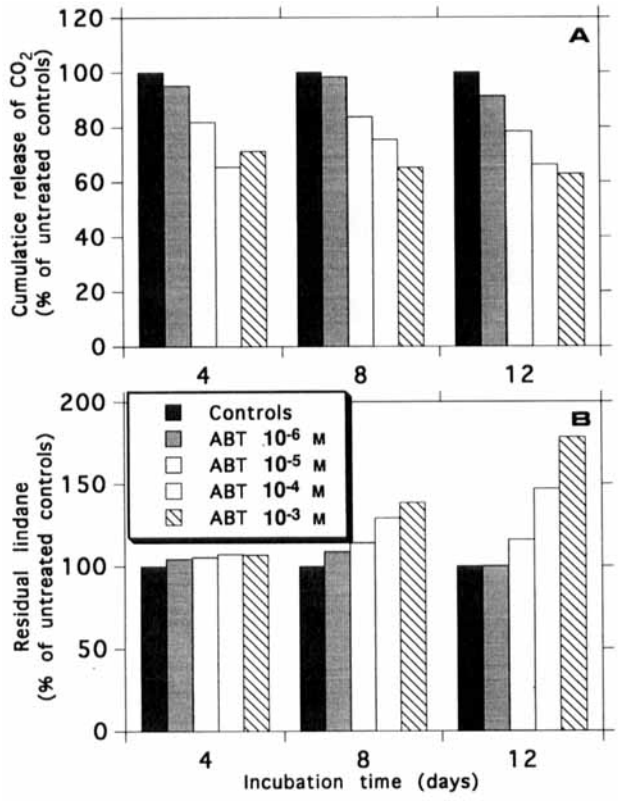

Fig. 7. Effects of aminobenzotriazole on (A) mineralization and (B) residual lindane content in medium from liquid cultures of *Phanerochaete chrysosporium*.

PB was assayed on cultures of *P. chrysosporium* at 10^{-3} and 10^{-2} M. Both concentrations induced moderate inhibitory effects on lindane mineralization noticed at day 4. Pesticide mineralization was identical in both PB-treated and control cultures after eight and 12 days of incubation (data not shown). The residual lindane in the medium was slightly increased by PB treatment after eight and 12 days of culture, but was unaffected during the early days of the experiment. In general, PB treatments modified the transformation of lindane to polar metabolites *a* and *b*. They also reduced TCCH amounts after eight and 12 days of culture, TCCE and TCCOL amounts being increased (Table 1).

Both modifiers were themselves without effect on fungal growth (data not shown).

4 DISCUSSION

The results presented in this paper confirm those previously reported.[13,14] They show that the white rot fungus *P. chrysosporium* transforms the insecticide lindane and partially mineralizes the molecule. We have characterized for the first time the main lindane metabolites from *P. chrysosporium* liquid cultures. Unfortunately, the small amounts of material available have prevented any detailed scheme of lindane transformation in *P. chrysosporium* cultures. Nevertheless, we suggest TCCH, TCCE and TCCOL structures which agree with published studies in vertebrates or microorganisms.[5–9,24,25]

Although there is a general consensus among investigators that the LDS is responsible for pollutant transformation, direct proofs with purified enzymes are rarely presented, and indirect data are not convincing. However, new hypotheses have now been reported. For example, the ability of *P. chrysosporium* to mineralize DDT was shown to be independent of the formation of the LDS.[26] Then, many researchers postulate that other fungal enzymatic systems can catalyse xenobiotic degradation reactions as an alternative or a complement of the LDS.[27,28] Numerous data also suggest that both

TABLE 1
Effects of Aminobenzotriazole (ABT) and Phenobarbital (PB) on Metabolite and Lindane Contents of Medium from Liquid Cultures of *Phanerochaete chrysosporium* after 12 Days of Incubation

Incubation conditions	*Unretained fraction*	*a + b*	*TCCOL + TCCE (c)*	*TCCH (d)*	*Lindane*
		(% of radioactivity in medium)			
Untreated controls	34·3	8·5	3·1	4·3	49·8
ABT (10^{-3} M)	11·6	0·0	0·0	0·0	88·4
PB (10^{-2} M)	37·9	1·1	5·2	0·0	55·8

intra- and extracellular enzymes may be sequentially involved in the degradation process of organic compounds, as recently reported in the case of chlorophenols.[29]

In this paper, we show that the main first steps of lindane degradation may not involve LiPs and MnPs. These results complement previous studies with purified enzymes acting under reducing conditions.[11] Increasing the oxygen level and the temperature in cultures has a strong activating effect on the rate of production of LiPs and MnPs, as has also been described for the rate of lignin degradation.[30] On the other hand, no strong differences were found in the rates of mineralization and transformation of lindane when the fungus was grown under 100% oxygen versus atmospheric conditions. Similar results have been reported previously in the case of DDT.[31] A comparison between Figs 5 and 6 does not show any correlation between pesticide mineralization/transformation and LiPs/MnPs production. Lindane transformation is maximal when peroxidase production (especially MnPs) is low. Conversely, it is lower under classical optimal ligninolytic conditions.

In living organisms, detoxication processes are mainly mediated by P450s. Such enzymatic systems are poorly known in micro-organisms, and have never been described in *P. chrysosporium*. Lindane undergoes a reductive dechlorination by vicinal dihaloelimination to yield TCCH. It is now established that the first step in biological breakdown of lindane is reductive in various cases. That reaction (probably catalyzed by P450s) occurred with rat liver microsomes and NADPH,[9,32,33] and with cell-free extracts of bacteria.[34] The subsequent steps involve oxidation reactions. TCCH is oxidized to TCCOL by microsomal fractions from both rat liver and house fly abdomen.[9,25] It requires NADPH and is inhibited by classical P450 inhibitors (carbon monoxide, piperonyl butoxide and SKF-525A). Little is known on the enzymatic systems involved in fungi.

Although appearing contradictory, our results obtained in *P. chrysosporium* cultures using classical P450 modifiers are consistent with the involvement of these systems in one or more steps of lindane metabolism. ABT inhibits the formation of all the isolated metabolites. In that case, a slight carbon dioxide release is still observed. On the other hand, PB does not affect lindane mineralization, yet it seems to modify the profile of isolated metabolites, with an increased oxidation of TCCH to TCCE and TCCOL. The distinct effects of the modifiers on lindane transformation can be explained. ABT is a mechanism-based inactivator efficient on numerous P450 isoforms. In our experiments, it inhibits the enzymes involved in the first steps (dechlorination and oxidation) of lindane transformation. The lack of metabolites is then responsible for the inhibition of mineralization. Conversely, P450 inducers are known to be highly specific for P450 gene families. For that reason, PB may be without notable effects on the isozymes involved in the first step of lindane breakdown (dechlorination), although stimulating the following reaction (oxidation). Nevertheless, an important step for lindane mineralization (ring opening) may not involve P450 enzymes. It remains unaffected by P450 modifiers, and may not be stimulated by high amounts of metabolites. A similar lack of effect of PB on chlorotoluron oxidation has been already reported[35] in wheat cell suspension cultures, although the transformation reactions were P450-mediated.[36] Moreover, we cannot exclude a rapid transformation of PB by the fungus, before exertion of any strong inducing effect.

Our results, as well as those from many other laboratory studies with fungal cultures have yielded an extensive list of xenobiotics susceptible to degradation by *P. chrysosporium* in liquid culture conditions. The results presented above clearly establish that the fungus is able to metabolize lindane without involving LiPs and MnPs. For the first time, they allow us to postulate that P450s can be active systems for pesticide detoxication in *P. chrysosporium*.

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